

August 28, 1949.

- a) Large series of lac- and Mal- mutants obtained from W1069 and W1077 ca 8/25. ( $w_{1140} = \text{Lys-Me-Tyro-Lac-}$ ;  $w_{1141} = \text{Hist-DdeVal-Mal-}$ ).
- N28. Mix  $w_{1140}$ ,  $w_{1141}$  in Penassay tubes.  
N30. Wash and plate on T/0; EMS Lac  
P31. 1 colony/15 plates.  
 streaked out on T/0 agar. 4 s.c. picked and streaked on lac; Mal EMB.  
 Non-coliforms dominate, but contamination with a lac+ Mal+ seen.  
 Purify and test nutrition.

These derivatives of W1045 show no signs of recombination.

Hfr crosses.

602

August 28, 1949.

Mix heavily in Petri assay:

A) W1059 + W814

B) W1059 + W1084

A). Malac ~~5~~  $\times 100$ . 13% Malac -  $3/500$  Malac  $\vee$ .  
Test on other sugars, ~~not~~ T1.

Lac  $25 \times 100$ .  $>1\%$  Lac  $\vee$ .

Mal+ "tested on lac"  
Mal-  $33 L- 2 L+$   
 $10 L- 50 L+$  (1 or 2 visible.) were  $\circled{B}$ .

Mal+ Xyl+

B) Malac  $10 \times 100 = 1000$  No Malac-!

Mal  $5 \times 100 = 500$ . 6 Mal  $\vee$  ?

Lac  $25 \times 150$

Mal+: 56 Lac-. (from Malac platings, all  
33 Lac- 2 lac+ Mal- are lac+).

Mal+: 89 lac- 2 lac+

A) loc & colonies

602A cont

L+ : 50: All 2nd - (?), All Xylo-, 14 el-,

337+ : 102+

4

Mattorescoring ~~is indefinite~~ because of fading  
in 1% of all ETBs.  
Use 1.5% hereforth to avoid this  
difficulty.

Random isolations show 77% L+ X- etc. -  
11% L- M+ 11% L- M-

~~#~~ September 2, 1949.

## syll tests

	Lic	Hac
1.	-	-
2.	-	-
3.	#-	+
4.	-	+
5.		++
6.		++
7.		++
8.		++
9.		++
10.		++
11.	-	+
12.	-	-
13.	-	+
14.	-	-
15.	-	+
16.	+	-
17.	-	-
18.	-	-
19.	-	-
20.	-	+
21.	+, -	+
22.	-, -	+
23.	-	+
24.	-	-
25.	-	+
26.	-	-

of 25 vac v,

10wue Xgl-Lac-

15

Xgf - Lac +.

Arizona

(Seq. Cols. 10) <sup>Rec.</sup>

Whole  
pop.

24

34 | 40

Jan.

35

Hfr selection

603

Sept 2, 1949.

602A = mixture of lac-Mal-recombinant colonies (5).  
Grow overnight in ~~E~~ Pernassay.

## A) Assay cultures

	$\times 10^7 \text{ ml}^{-1}$	$\bar{m}$
602A	2.5	3
K-12	42.64	53
1033	20.27	23
58-161	40.56	48
Y10	35.33	34.

Sept. 2.

B) Inoculate .001 ml 602A + following: into Pernassay tubes.

A K12 1ml	46+/59-	31+/27-
B W1033 .5ml + Y10 .5ml	213+/0-	136+/0-
C 58-161 .5ml + Y10 .5ml	71+/2-	156+/3-

Plated at  $\approx 10^{-7}$ 

Sept 5, 1949.

Lact Mal

B. 17 Lactu. Test +, - or Maltose. (5 each)

1-16 parents only.

#17. 4 Lact+Mal-, 1 Lact+Malt+, 5 Lact-Malt+

September 7, 1949.

Graduate W466 and W477 on EMB Mal 7 sec. 44.  
25 plates each. ca 200/

W466 12 mutants purified. #3 is glucose -  
All but #3 and #10 are maltoose slow. T.O.

#3 Mal - Glu -  
#10 Mal - Glu +

1187?

W477 16 mutants purified.

#3, 7, 13 are glucose -. #2 is "thin".

#5, 1, 2, 8, 11, 14 are maltoose slow

#12 forms minute colonies.

1178-1188

#3 Glu -  
4  
5  
6  
7 Glu -  
9  
10  
12  
13 Glu -

check for 30° fermentation of Glu -. None were temp. sens.

September 15 ff. 1949.

as 604. W466. 20 Mal EMB plates; 200/ = 4000.

# 1-8 are slow or nearly - fermenters of maltose. Test in Glu

# 9, 10 are Mal -. Streak out. (Glu+) ; W1208-1209

# 11 as streaked from 1st isolation had mostly Mal-(slow) colonies, but 3 colonies sectoring on maltose. [These may be suggested to

be  $\frac{\text{Mal}+ \text{ Lethal}}{\text{Mal}-}$  ]. No Mal+ were seen.

Streak out on Mal EMB.

N18 When streaked out, Mal<sub>v</sub> colonies above gave mixtures of pure + and - and no apparent Mal<sub>v</sub>. One possible Mal<sub>v</sub> (more likely conglomerated) was noted. Streak out: These colonies are very difficult to interpret, mainly because there were no pure + colonies on the original plate. Conceivably, there had been induced an unstable intermediate allele which usually shifted to Mal- but rarely (i.e., within 3 colonies) reverted to Mal+.

## Test cross for Mal,-

610

September 13, 1949

(cont.)

W1178 - 1183 x W1014 }  
 Mal EMS(B<sub>1</sub>).  
 W1187 x W814

Mal+/total prot.

1187 20% +

1178 0/100

MAL<sub>1</sub>

1179 30%

1180 1/3 (ca 20% prot.)

1181 80% +

1182 30% +

1183 0/100

MAL<sub>1</sub>

677 SR X 478

= W1177

cultures  
purified

612

M. Donbrot

culture	from	Malt+	Streptomyces	Lactose			Lac+	Lac-	Total.
1	malt synth	+	-	-	Strep resistant	malt+	0	2	2
2	"	+	-	+	malt-	4	5	9	
3	"	+	-	-					
4	"	+	-	-					
5	"	+	-	-	Strep sensitive	malt+	13	6	19
6	"	+	-	+	malt-	0	0	0	
7	"	+	-	-					
8	"	+	-	-					
9	"	+	-	-					
10	"	+	-	-					
11	"	+	-	-					
12	"	+	-	-					
13	"	+	-	-					
14	"	+	-	-					
15	"	+	-	-					
16	from Malt Synth + B'	+	-	-					
17	"	+	-	-					
18	"	+	-	-					
19	"	+	-	-					
20	"	+	-	-					
21	"	+	-	-					
22	"	+	-	-					
23	"	+	-	-					
24	"	+	-	-					
25	"	+	-	-					
26	"	+	-	-					
27	"	+	-	-					
28	malt. synth	+	-	-					
29	"	+	-	-					
30	"	+	-	-					

Total organisms testedTested for lactoseLac+      Lac-

Strep sensitive. Malt+    30      26      16      10  
 Malt-                    0      0      0      0

Strep. resist. Malt+    8      5      1      4  
 Malt-                    44     36      6      23

38    4431    36.    17    6    14    23

612 a

H.D.

Cross: 677 SR (1177) X 478.

4 parent diploids selected, Segregated

Original diploid	Segregant	Lactose	Synth	Resistant Streptomycin			
1	1	-	-	+			
	2	-	-	+			
	3	-	-	+			
	4	-	-	+			
	5	-	-	+			
	6	-	-	+			
	7	-	-	+			
	8	-	-	+			
	9	-	-	+			
	10	-	-	+			
	11	-	-	+			
	12	-	-	+			
	13	-	-	+			
	14	-	-	+			
	15	-	-	+			
	16	-	-	+			
	17	-	-	+			
	18	-	-	+			
	19	-	-	+			
	20	-	-	+			
	21	-	-	+			
	22	-	-	+			
	23	-	-	+			
	24	-	-	+			
	25	-	-	+			
	26	-	-	+			
	27	-	-	+			
	28	-	-	+			
	29	-	-	+			
	30	-	-	+			
	31	-	-	+			
	32	-	-	+			
	33	-	-	+			
	34	-	-	+			
	35	-	-	+			
	36	-	-	+			

Diploids grew on streptomycin EMB, showed segregation into lac+ and lac- all growing on streptomycin. Diploids could be isolated from streptomycin plates.

\*Some colonies grew

??

Hence: streptomycin resistance is dominant, recovered in all segregants for lactose fermentation & nutrient deficiencies. sensitivity is lost in cross. Heterozygous

677 SR x 478

~~Cultures  
not purified~~

b12b  
M. Rodanoff

Prototrophs	Streptomyces		Lactose	Maltose	Streptomyces		Lactose	Maltose
	Synthetic	Synthetic	+	-	Synthetic	Synthetic	+	-
1	+	+	-	-				
2	+	-	-	-	Prototroph	25	+	-
3	-	+	-	-	26	+	-	-
4	+	-	-	-	27	+	-	-
5	-	-	-	-	28	+	-	-
6	+	-	-	-	29	+	-	-
7	+	-	-	-	30	+	-	-
8	+	-	-	-	31	+	-	-
9	-	-	-	-	32	+	-	-
10	+	-	-	-	33	+	-	-
11	(colonies)	-	-	-	34	+	-	-
12	+	-	-	-	35	+	-	-
13	-	-	-	-	36	+	-	-
14	+	-	-	-	37	+	-	-
15	-	-	-	-	38	+	-	-
16	+	-	-	-	39	+	-	-
17	-	-	-	-	40	+	-	-
18	-	-	-	-				
19	+	-	-	-				
20	+	-	-	-				
21	-	-	-	-				
22	+	-	-	-				
23	-	-	-	-				
24	+	-	-	-				
<i>Malto-</i>								
1	+	-	-	-				
2	+	-	" no gr.	-				
3	-	-	" "	-				
4	-	-	" "	-				
5	(colonies)	-	" no gr.	-				
6	+	-	" "	-				
7	-	-	" "	-				
8	-	-	" "	-				
9	-	-	" "	-				
10	-	-	" "	-				
11	-	-	" "	-				
12	-	-	" "	-				
13	-	-	" "	-				
14	-	-	" "	-				
15	-	-	" "	-				
16	-	-	" "	-				

W 108 mutations occurring spontaneously on nutrient agar.

Strain isolated from: Gluc Galact malt. lact. xylose manitol Tech

1	Glucose	+	S-	-	S-	-	-	-	-
2	"	+	G-	-	G-	-	+	-	-
3		+	G-	-	G-	-	*	-	*
4		+	G-	-	G-	-	*	-	*
5		+	G-	-	G-	-	*	-	*
6		+	G-	-	G-	-	*	-	*
7		+	G-	-	G-	-	*	-	*
8		+	G-	-	G-	-	*	-	*
9		+	G-	-	G-	-	*	-	*
10		+	G-	-	G-	-	*	-	*
11	Maltose	+	S-	-	S-	-	-	-	-
12		+	S-	-	S-	-	-	-	-
13		+	S-	-	S-	-	-	-	-
14		+	S-	-	S-	-	-	-	-
	Galact	(S-)							
	Lactose	S-							

D. D.

9-15-49 613

## Fermentation tests on Shaprio's cultures.

Het in-crosses.

614

Sept. 16, 1949.

A.	58-161 x W1178	* 6 tests; all Lac++; 96 tests. 1 Lacv	2?
B.	58-161 x W1183	52 tests. 2? Lacv; 60 tests	6 Lacv 2?
C.	WY78 <sup>Mal+</sup> x W1178 <sup>Mal-</sup>	100 tests. 4 Lacv	
D.	WY78 x W1183	52 tests. 1 Lacv	

A. 72, 3 (?)

B. 1 het - lacv Mal++

614-B1

C. 1-4 lacv Mal++

614-C:1-4

D. 1 lacv Mal++

614-D1

[#1 and 2 throw off frequent lac- prototrophs].

B. 1-6 lacv - 7, 8 Lacv?

5, 6, 7 are Mal+, - 8 no Mal+ Lac++.

1-4 are Mal-

\* M.O.  
Misinoculated

See 618

September 17, 1989.

	on EMS Lac;	EMS Mal.				
A. W1178	x W828	52 tests	1?? Lac <u>v</u>			
B. W1178	x W836	100 tests	145 Lac <u>v</u> 6-7?			
C. W1178	x W760 very infertile	62 tests	122 Lac <u>v</u> 6 tests. 2 Lac <u>v</u> .			

In 615B, both on Mal EMS and Lac EMS, - colonies seem to grow better than +! stick out from Mal EMS:

A	Mal -					
B	1-4	Mal --	: 5: Mal+ ( <u>v</u> ??); 6 M+, -	7 Mal+		
	All Lac <u>v</u> .		Isolated Lac <u>v</u> of 5, 6 were	(Redeem or reabsorb)		
C	1-2 Mal -		pure Mal+, 14 Mal - resp			

See 618

## New coli crosses.

September 17, 1979

D. W1189 x W1195 [A.C. 1 Valting Mal- x Lac Trypt Lac- 2]

E. W1189 x W1205 [A.C. 1 Valting Mal- x Thre Hist Lac- 3]

F. W1195 x W1191 [Lac Trypt Lac- 2 x Thre Hist Mal- 4]

Controls: Wash cultures from 92. Conc. 5x. Use 1 ml/plate

1 (W1189) 4 colonies / 4 plates Lac + Mal -

2 (W1195) 0 " 1 "

3 (W1205) 0 " 1 " Lac - Mal +

4 (W1191) 8, 12, 16, 11 / 4 plates ca. 12/plate!

D. 2 colonies / 9 T(0) plates 2 Lac + / 4 EMS Lac plates

E. 7 colonies / 9 T(0) plates 1 Lac + 12 "

F. 10, 6, 2 / T(0) plates

W1189 and W1191 appear to be exceptionally mutable.

Their nutrition should be carefully checked.

P19 Tests on "cross" prototrophs

D: 1-4 Mal + Lac +

E 1, 6 + -  
2-5, 7 + +

F 1-16 + +

These prototrophs are clearly either contaminants or recombinants, probably former. Parents had been checked on EMB and found pure +.

A20 Tests on "reversion" prototrophs

1 Mal + Lac +

4 Mal + Lac +

} must be contaminated !!

1	0, 2, 2	1113:	The Hist. x	Val Arg.
2	1, 2, 2	1113 x 1114	"	Val-Dos, Arg.
3	2, 1, 4	1113 x 1115	"	Leuc Trapp.
4	0, 0, 0 sm sm	1113 x 1114	Val Arg	Hist Leuc
5	0, 2, 1	1113 x 1115	"	The Hist
6	1, 2, 0	1114 x 1114	Val Dos Arg	Hist Leuc
7	2, 0, 0	1114 x 1115	"	The Hist
8	7, 4, 8	"	"	Leuc Trapp.
9	0, 0, 0	1115 x 1115	The Hist	"
10	>15.	1113 1115	Val Arg	The Hist

Prototrophs occur amidst rather heavy synethophenia!

Pick colonies from #8, #10. - streak on T(0).

Each of 12 tested from #8 and #10 grew out as single colonies on T(0), and were further picked to EMB Lac, Mal, X-gal on which they agreed with their parent in being ++.

September 25, 1949.

Collect following heterozygotes :  $4 = 614$   $5 = 615.$

$$5A = 1178 + 820$$

$$5B = 1178 + 836$$

$$5\% = 1178 \times 760$$

$$4A = 58 - 161 \times 0.1178$$

$$4B = 88 - 161 \times 0.1183 \\ 4D = 0.478 \quad "$$

$$4D = \omega + \gamma x$$

—  
—  
—

MENSHE

614  
A.

2	Lact	Malt	{	Resol!
B	Lact	Malt		
	Lact	Malt		

०००

0	<sup>1?</sup> 25 Lac V	Mal + or -??	
1	Lac slow	Mal -	
2	Sac slow	Mal -	
3	Lact +	Mal -	
4	<del>Lact</del> V	"	
5	Lact	"	
6	Lact	"	

6

Lac V	Mal v?
"	Mal +
"	Mal v
"	Mal +

D

Lac v Malt+

615

B | 5 (4 tests) #1, 4 Lac<sup>+</sup>; All Malt<sup>+</sup> but #1 shows ~~nothing~~<sup>+</sup> on Malt.

6 (4 kids) All Lac<sup>v</sup> Mal- (segregating blue + white) Use for Rev  
6<sub>o</sub> (1 kid - broad streak) Many Lac<sup>v</sup> Almost completely Mal-.

615B5 = Lac v Mal+

See 615 for data on other laws.

615 B6 - Lactu Mal -

Sept. 23, 1949.

W466 Mal<sub>x</sub>- × W677.4 plates Mal<sub>E</sub> MS

A) W1208 × W677.

2 Mal<sup>+</sup> / 600 Mal -.

In view of rarity of Mal<sup>+</sup>  
in W466 × W677, these low  
frequencies do not necessarily  
speak for close linkage.

B) W1209 × W677

5 Mal<sup>+</sup> / 600 Mal -

Streaks out + prototrophs:

1) Pure Mal+

2) 3 Mal<sub>v</sub>, 4 Mal<sub>+</sub>.Reisolate [to use for lac reversions  
studies].

*Coli new crosses.*

620

September 28, 1959.

Bacteria grown together 48 hours. Plate ex. .5 ml  $\div^0$  / plate 5/10).

Inc. 48 hours.

A W1189	0	0	0		Lac+M - Valtex Sh 3
B D W1191	0	0	0	0	Lac+M - Th Hist Sh 1
C E W1195	0	0	0	0	East? L-M + Lac Try Sh 1
F W1205	(5)	(1)	(5)	0	L-M + $\Delta^3$ P Th Hist $\Delta^3$ P Prototrophs grew more poorly on T(0) agar than those below.
G W1189-1195	2	3	5	4	
H W1189-1205	3	1	2	0	
I W1195-1191	0	0	0	0	

Picks, delete and test on Lac; Mal:

E 2	Lac - Mal+
F 10	All Lac - Mal+
G 12	All Mal+; 8 Lac - 4 Lac+
H 6	Lac+ Mal+
	Lac - Mal+

These results strongly suggest recombination between W1189 and either W1195 or W1205. However, there is a curious instability of the individual parents. The Lac+ Mal+ prototrophs are, however, unique.

Re-purify and re-test parents!